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論文内容の要旨

Introduction

Bacterial infections and cancers are the leading causes of death in the world. Therefore, the development of new and effective drugs plays an important role to combat infection and cancer. Many marine organisms have been explored for the pharmaceutical field because of the possibility of identifying new bioactive compounds. Among them, marine sponges have been recognized not only as a prolific source of compounds with novel chemical structures, but also as a source of bioactive metabolites with pharmaceutical values. About 30% of all marine natural products have been isolated from sponges. Therefore, marine sponges continue to attract attention as potential resources for new biologically active compounds. However, there are not many reports for investigation of chemical constituents in Vietnamese marine sponges. In order to discover new antibacterial and anticancer secondary metabolites, I collected seven species of marine sponge in Vietnam. Out of seven species, only three species namely *Spongia* sp., *Xestospongia testudinaria*, and *Clathria reinwardti* were selected for further studies because they were enough amounts to conduct the isolation of compounds.

1. Constituents of *Spongia* sp. and their antibacterial and cytotoxic activities

Merosesquiterpenes with anti-inflammatory, antibacterial, antiviral, and antitumor activities have been isolated from the marine sponge of the genera *Dysidea*, *Spongia*, and *Dactylospongia*. Further investigation of the EtOAc extract of *Spongia* sp. led to the isolation of 22 compounds including 9 new merosesquiterpenes langcoquinones A (**1**), B (**2**), langconols A-C (**10**–**12**), langcoquinone C (**13**), langcoquinones D-F (**14**–**16**) and 13 known compounds, dictyoceratin A (**3**), polyfibrospongol B (**4**), ilimaquinone (**5**), smenospongine (**6**), 19-hydroxy-polyfibrospongol B (**7**), smenospongidine (**8**), and nakijiquinone L (**9**), smenospongorine (**17**), polyfibrospongol A (**18**), dictyoceratin C (**19**), 8*E*-pentacosen-4-ynoic acid (**20**), indole-3-carboxylic acid (**21**), and a mixture (1:1) of cholesterols 5α,8α-epidioxycholesta-6,22-dien-3β-ol and 5α,8α-epidioxycholesta-6-en-3β-ol (**22**) (Figure 1). The chemical structures of the isolated compounds were elucidated by analyses of their spectroscopic data including 1D and 2D NMR, HREIMS, and by comparison with published data.

The isolated compounds from *Spongia* sp. were evaluated for their antibacterial and cytotoxic activities [1-3] (Table 1). Among the isolates, sesquiterpene quinones (**1**, **2**, **5**, **6**, **8**, **9**, **13**, **14**, and **17**) exhibited strong antibacterial activities against Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* with MIC values ranging from 6.3 to 25.0 μM. Furthermore, these compounds also had cytotoxic activities against the three cancer cell lines (A549, lung cancer; MCF7, breast cancer; HeLa, cervical cancer) and a normal cell line (WI-38, fibroblast) with IC_{50} values ranging from 3.0 to 9.9 μM. Remarkably, langconol A

(**10**) exhibited potent antibacterial activities against *B. subtilis* with an MIC value of 12.5 μM without showing cytotoxicities against the human cancer and normal cell lines. A similar case was also found with dictyoceratins A (**3**) and C (**19**), which showed the activities against both *B. subtilis* and *S. aureus* with MIC values of 6.3 μM, whereas both compounds did not show any cytotoxicities against the tested cell lines.

The structure–activity relationship (SAR) of sesquiterpene quinones (**1**, **2**, **5**, **6**, **8**, **9**, and **13**–**17**) on antibacterial and cytotoxic activities suggested that the presence of quinone moiety was crucial for biological activities. Furthermore, SAR study also suggested that the presence of the hydrophobic side chains at C-20 enhanced antibacterial activities as deduced from comparison of the structures of (**1**, **2**, **5**, **6**, **8**, **9**, **13**, **14**, and **17**) with those of **15** and **16**. Moreover, the results of cytotoxic activities followed the same pattern as those of antibacterial activities: sesquiterpene quinones with hydrophobic side chains (**1**, **2**, **5**, **6**, **8**, **9**, **13**, **14**, and **17**) exhibited significant cytotoxic activities than those with less hydrophobic side chains (**15** and **16**). Regarding to sesquiterpene phenols (**3**, **4**, **7**, **10**–**12**, **18**, **19**), the presence of the methyl group at C-14, the hydroxyl group or no substituent at C-18, and the absence of the substituent at C-19 contributed to possess good antibacterial activities. These observations thus suggested that sesquiterpene quinones could be potential candidates for antibacterial and cytotoxic activities.

2. Constituents of *Xestospongia testudinaria* and their antibacterial and cytotoxic activities

X. testudinaria belongs to the family Petrosiidae. The genus *Xestospongia* is one of the most widespread in several regions, and has been recognized as rich sources of different chemical classes such as isoquinoline, macrocyclic quinolizidines, pyridoacridine alkaloids, quinones, polyhydroxy sterols, brominated polyacetylenic acids, and esters. A new sterol langcosterol A (**23**), together with four known compounds, 24-methylene-26,27-dimethylcholest-5-en-3β-ol (**24**), 24-hydroperoxy-24-vinyl cholesterol (**25**), 4-hydroxybenzoic acid (**26**), octadeca-9*E*-17*E*-diene-7,15-diynoic-18-bromomethyl ester (**27**), were isolated from the EtOAc extract of Vietnamese *X. testudinaria* [4] (Figure 2). Their chemical structures were determined by means of spectroscopic analyses including 1D and 2D NMR and HREIMS, and by comparison with published data. All of the isolated compounds did not show any antibacterial activity against *B. subtilis*, *S. aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. In contrast, **23** and **25** exhibited moderate cytotoxic activities against the three cancer cell lines and the normal cell line with IC_{50} values ranging from 29.0 to 70.0 μM.

3. Constituents of *Clathria reinwardti* and their antibacterial and cytotoxic activities

C. reinwardti belongs to the family Microcionidae. Previous chemical investigations of genus *Clathria* have reported that this genus contains the various types of secondary metabolites including thiosugars, alkaloids, terpenes, and sterols. To the best of our knowledge, chemical constituents of a Vietnamese marine sponge *C. reinwardti* have not yet been investigated. Eleven compounds including nine steroids, cholest-4-en-3-one (**28**), cholest-5-en-3-ol (**29**), cholest-5-en-4-ol (**30**), cholest-3-one (**31**), 24-hydroxy-24-vinyllathosterol (**32**), cholest-5-en-3,7-diol (**33**), 4,5-epoxy-3α-cholesterol (**34**), 6β-carboxaldehyde-B-norcholestan-3β,5β-diol (**35**), cholestan-3,3-dimethoxy (**36**), one indole alkaloid, indole-3-carboxylic acid (**37**), and a brominated phenolic derivative, 2-(2′,4′-dibromophenoxy)-4,6-di-

bromophenol (**38**), were isolated from the EtOAc extract of *C. reinwardti* (Figure 3). Compounds **35** and **36** were isolated for the first time from a natural source, while **38** was isolated from this genus for the first time. The structures of the isolated compounds were elucidated by analyses of their spectroscopic data and by comparison with the reported literature. Compound **38** exhibited significant antibacterial activities against both Gram-positive bacteria *B. subtilis* and *S. aureus* with MIC values of 0.5 μM and Gram-negative bacteria *E. coli* and *K. pneumonia* with the same MIC values at 3.3 μM. Furthermore, **38** also had good cytotoxic activities against four tested cell lines with IC_{50} values ranging from 5.2 to 10.2 μM. Considering the SAR study of the isolated compounds, the 3*β*,5*β*-diol and the aldehyde functional groups might play an important role for cytotoxicity. In addition, brominated phenolic derivative **38** is also an attractive compound for further study related to antibacterial and cytotoxic activities.

Conclusion

The investigation of the chemical constituents of the EtOAc extract from three species of Vietnamese marine sponges *Spongia* sp., *Xestospongia testudinaria*, and *Clathria reinwardti* led to the isolation of 38 compounds (**1**−**38**) including ten new compounds (**1, 2, 10**−**16** and **23**), and 28 known compounds (**3**–**9** and **17**–**22**). The isolated compounds in this study belong mainly to three kinds of different skeletons including sesquiterpenes (**1**−**19**), steroids (**23**−**25** and **28**−**36**), and phenolic derivatives (**26** and **38**). The bioassays suggested that most of the sesquiterpene quinones (**1**, **2**, **5**, **6**, **8**, **9**, **13**, **14,** and **17**) were attractive candidates for both antibacterial and cytotoxic activities, while most of the sesquiterpene phenols (**10**, **3**, and **19**) were favored to antibacterial activities. The brominated phenolic compound **38** also will be interested in further study due to the potential activities. Furthermore, the bioassay suggested that the presence of more than two functional groups at the A and B rings in steroids may enhance cytotoxicity. This study thus provided insight into not only the structural diversity, but also antibacterial and cytotoxic secondary metabolites in Vietnamese marine sponges.

References

1. Nguyen HM, Ito T, Win NN, Kodama T, Hung VQ, Hoai NT, Morita H (2016) New antibacterial sesquiterpene aminoquinones from a Vietnamese marine sponge of *Spongia* sp. *Phytochem Lett* 17:288–292.
2. Nguyen HM, Ito T, Kurimoto S, Ogawa M, Win NN, Kodama T, Hung VQ, Hoai NT, Kubota T, Kobayashi J, Morita H (2017) New merosesquiterpenes from a Vietnamese marine sponge of *Spongia* sp. and their biological activities. *Bioorg Med Chem Lett* 27:3043–3047.
3. Ito T, Nguyen HM, Win NN, Hung VQ, Hoai NT, Morita H (2018) Three new sesquiterpene aminoquinones from a Vietnamese marine sponge of *Spongia* sp. and their biological activities. *J Nat Med* 72:298–303.
4. Nguyen HM, Ito T, Win NN, Hung VQ, Hoai NT, Morita H (in press) A new sterol from the Vietnamese marine sponge *Xestospongia testudinaria* and its biological activities. *Nat Prod Res*. DOI: 10.1080/14786419.2018.1465057. In Press.

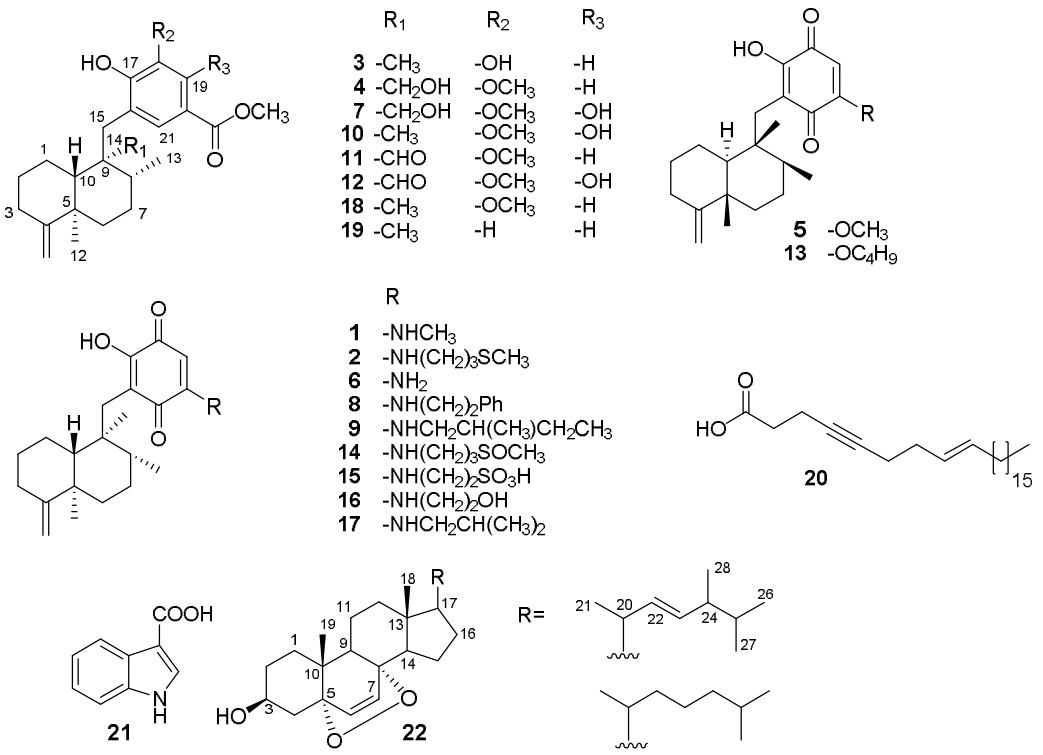

Figure 1. Structures of isolated compounds **1**–**22** from the EtOAc extract of *Spongia* sp.

Table 1. Antibacterial and cytotoxic activities of isolated compounds **1**–**21**

Types of skeletons	Compds	Antibacterial activity MIC (μM)		Cytotoxic activity IC_{50} (μM)			
		B. subtilis	*S. aureus*	HeLa	A549	MCF7	WI-38
Sesquiterpene quinones	**1**	12.5	12.5	8.1	9.9	7.9	8.4
	2	12.5	12.5	8.0	6.2	8.7	8.8
	5	6.3	6.3	7.6	5.9	8.3	9.7
	6	12.5	6.3	7.5	7.8	8.3	9.2
	8	12.5	12.5	5.0	4.0	6.5	3.0
	9	6.3	6.3	4.8	8.9	7.6	6.9
	13	6.3	12.5	7.6	6.6	9.6	8.2
	14	12.5	25.0	8.6	8.9	5.9	5.6
	15	-	-	-	-	-	-
	16	-	-	-	-	-	-
	17	12.5	25.0	7.6	6.7	4.6	3.5
Sesquiterpene phenols	**3**	6.3	6.3	-	-	-	-
	4	-	-	-	-	-	-
	7	-	-	-	-	-	-
	10	12.5	-	-	-	-	-
	11	-	-	-	-	-	-
	12	25.0	-	7.2	7.8	5.0	8.7
	18	-	-	-	-	-	-
	19	6.3	6.3	-	-	-	-
Fatty acid	**20**	-	-	-	-	-	-
Indole alkaloid	**21**	-	-	-	-	-	-
Positive controls	Amp	6.3	6.3				
	5-FU			5.3	4.7	5.6	6.8

- : >100 μM, Amp: Ampicillin, 5-FU: 5-Fluorouracil

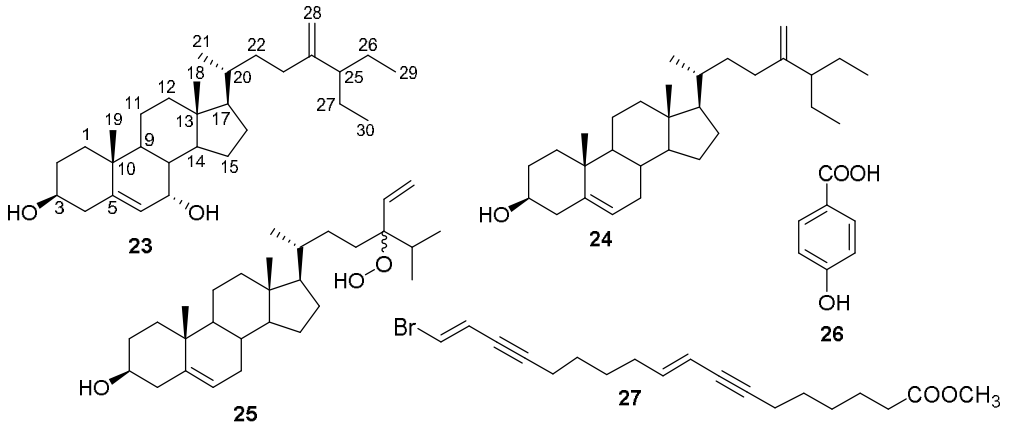

Figure 2. Structures of isolated compounds **23**–**27** from the EtOAc extract of *Xestospongia testudinaria*.

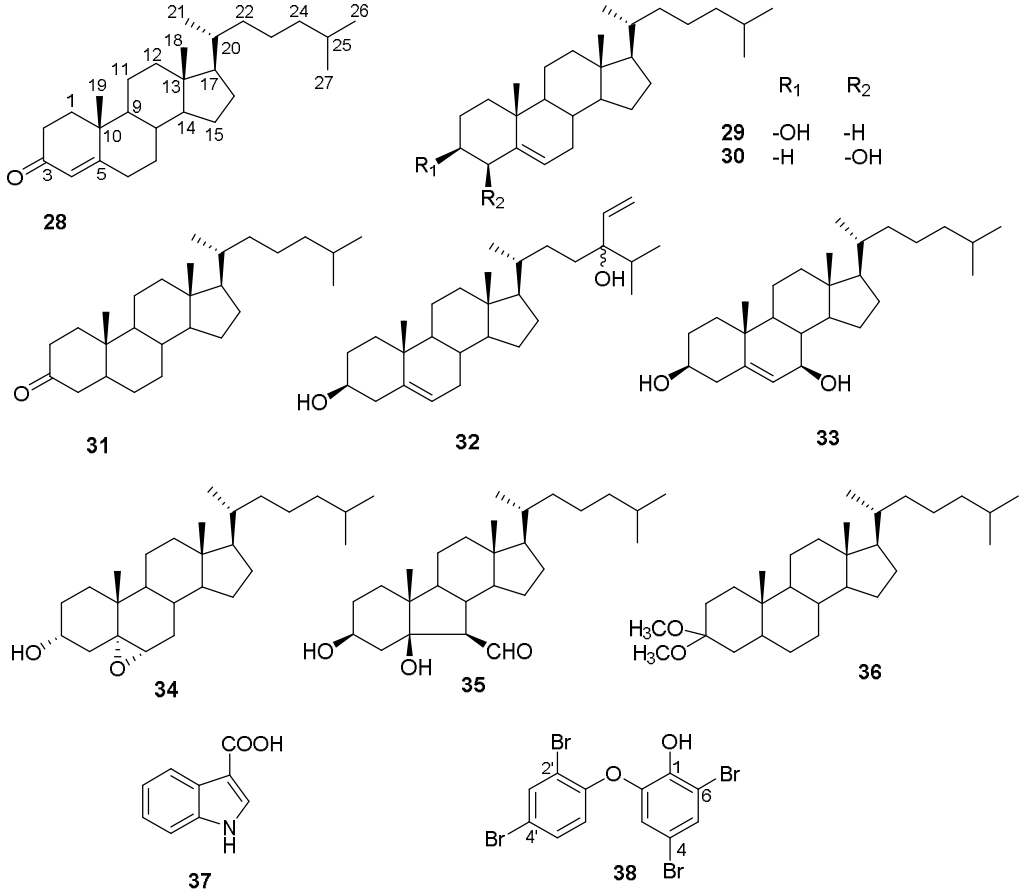

Figure 3. Structures of isolated compounds **28**–**38** from the EtOAc extract of *Clathria reinwardti*